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Small Round-Structured (Norwalk-Like) Viruses and Classical Human Caliciviruses in Southeastern Australia, 1980–1996

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A total of 6,226 fecal samples collected from 1980 to 1996 in the Australian states of Victoria, New South Wales, and Tasmania from individuals with gastroenteritis were tested for small round-structured viruses (SRSVs) and classical human caliciviruses (ClHuCVs) by electron microscopy. There were 223 samples positive for SRSVs, and nine positive for ClHuCVs. SRSVs were detected in individuals of all ages and were commonly associated with gastroenteritis outbreaks in nursing homes and hospitals. SRSVs were detected throughout the year, but were more common in the period from late winter to early summer in Australia (August to December). There were peaks of virus activity in the early 1980s and more recently in 1995 and 1996. Analyses by RT-PCR and sequencing of a segment of ORF1 encoding the putative RNA polymerase for SRSVs and ClHuCVs showed the presence of viruses belonging to several genogroups. Viruses of genogroup 1 (Norwalk/Southampton-like) and genogroup 3 (ClHuCVs) were relatively rare. Viruses of genogroup 2 (Snow Mountain-like) were common, and could be divided into two subgroups, one containing Toronto/Mexico-like viruses, the other Lordsdale/Camberwell-like viruses. The majority of viruses detected belonged to this latter subgroup. ***J. Med. Virol. 55:312–320, 1998.***

KEY WORDS: SRSV; enteric viruses; genetic variation

INTRODUCTION

Small round-structured viruses (SRSVs) is the name commonly applied to a group of viruses within the family Caliciviridae that are associated with gastroenteritis in humans [Caul, 1996a, 1996b]. Although the illness is usually mild and self-limiting, the virus is highly infectious, and a major cause of morbidity [Riordan, 1991; Taterka et al., 1992]. On some occasions, infection may also be associated with mortality [Gellert et al., 1990; Russo et al., 1997; Ryan et al., 1997]. The SRSVs can be distinguished by electron microscopy from classical human caliciviruses (ClHuCVs) which have typical cup-shaped depressions on their surface [Cubitt et al., 1994]. Infection by ClHuCVs also causes gastroenteritis, more commonly in children than adults [Greenberg and Matsui, 1992; Caul, 1996b].

A number of reports indicate that SRSV infections are quite common in Australia. In the late 1970s, it was shown that gastroenteritis associated with the consumption of oysters was due to SRSV infection [Murphy et al., 1979; Linco and Grohmann, 1980]. Other studies from our laboratories have shown an association between the detection of SRSVs and gastroenteritis in the state of Victoria in a variety of situations, including a guest house, a hospital, hostels for the aged, a center for mothers and babies, as well as school and recreation camps [Oliver et al., 1985; Cauchi et al., 1996; Marshall et al., 1997; Russo et al., 1997]. ClHuCV-associated gastroenteritis has also been documented in Australia [Grohmann et al., 1991].

Partial nucleotide sequencing of open reading frame 1 (ORF1) of the SRSV and ClHuCV genomes has provided a classification of the viruses based on the variation observed. For the SRSVs, two main genogroups have been identified: genogroup 1 (Norwalk virus group) and genogroup 2 (Snow Mountain virus group); viruses in genogroup 2 cluster into two subgroups [Caul, 1996a; Jiang et al., 1996]. The ClHuCVs are assigned to a third genogroup [Liu et al., 1995; Jiang et al., 1996]. Previous studies have identified genogroup 2 viruses in Victoria [Cauchi et al., 1996; Marshall et al., 1997], but no systematic study in Australia of the geno-

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groups of viruses collected over a prolonged period has been reported. We now describe an analysis of fecal samples collected for 17 years from 1980. These samples were predominantly from Victoria, with a few specimens from the neighboring states of New South Wales and Tasmania. We sought evidence for viruses representing the three major genogroups already described in the literature, and for changing patterns in the prevalence of the genogroups during the years since 1980.

MATERIALS AND METHODS

Fecal Samples

The virology department at the Fairfield Infectious Diseases Hospital (now the Victorian Infectious Diseases Reference Laboratory) is the major public health virus diagnostic and reference laboratory in the state of Victoria, Australia. The laboratory has monitored viral causes of gastroenteritis, chiefly in adults (i.e., individuals aged 16 years and over) since 1980. From 1980–1996, fecal specimens (6,106) from within Victoria were received from a variety of sources, including the Fairfield Infectious Diseases Hospital, other hospitals in Melbourne and country Victoria, the state Health Department, the Microbiological Diagnostic Unit, University of Melbourne, and private pathology laboratories. Specimens from the states of New South Wales and Tasmania were much fewer (74 and 46, respectively) and came from a number of pathology laboratories. Clinical notes accompanying the specimens indicated that the majority of specimens were from individuals with gastroenteritis; all specimens were presented for laboratory testing of gastroenteritis viruses. The presentation of specimens over the 17 years of this study reflected community concerns relating to SRSV gastroenteritis in southeastern Australia, particularly Victoria.

For the purposes of this study, an incident was defined as an occurrence of gastroenteritis, involving one or more individuals, for which a single original source was thought responsible. A sporadic incident was defined as one involving a limited number of individuals (usually no more than three). An outbreak was defined as involving a larger number of individuals (four or more), with person-to-person spread of the infection being a common occurrence.

Preparation of Fecal Samples and Electron Microscopy

Specimens were processed and examined for electron microscopy as previously described [Oliver et al., 1985; Cauchi et al., 1996]. Briefly, samples were clarified by two low-speed centrifugation spins, and then virus was pelleted by ultracentrifugation through a 45% (w/v) sucrose cushion. Concentrated virus was negatively stained with 3% phosphotungstic acid [Cauchi et al., 1996].

Reverse Transcription and Polymerase Chain Reaction

RNA was extracted from viral samples which had been concentrated by centrifugation and were positive for virus by electron microscopy. To extract RNA, samples were diluted to 100 μl in water and added to 340 μl of a solution containing 4 M guanidine isothiocyanate, 25 mM sodium citrate, 0.5% N-lauroylsarcosine, 100 mM 2-mercaptoethanol, and 350 mM sodium acetate. The mixture was extracted with 360 μl of phenol/chloroform (5:1) and RNA precipitated from the aqueous phase by the addition of 40 μg of glycogen and 300 μl of isopropanol. The precipitate was collected by centrifugation, washed in 500 μl of 80% ethanol, dried, and dissolved in 10 μl of water.

The procedures for reverse transcriptase-polymerase chain reaction (RT-PCR) using SuperScript™ II RNase H–reverse transcriptase (BRL, Life Technologies Inc., Gaithersburg, MD) and AmpliTaq® DNA polymerase (Perkin-Elmer, Norwalk, CT) were similar to those previously described [Cauchi et al., 1996]. Random hexameric primers were used for reverse transcription. To amplify short segments of ORF1 by PCR, the following primer pairs were used, with degeneracies shown in parentheses: genogroup 1, primers 2492 (modified primer 3: 5′-CACCATCTGAG[A/G]T[G/T]GATGT-3′) and 51 (5′-GTTGACACAATCTCATCATC-3′) [Moe et al., 1994]; genogroup 2, primers 2493 (modified primer 3) [Moe et al., 1994; Cauchi et al., 1996] or 4779 (5′-CACCAAGTGTG[A/G]T[G/T]GATGT-3′, 5′-CACCAAGTGTG[A/G]T[G/T]GATGTGGG-3′, respectively) and primer 51. Primer 51 was later modified to 5′-GTACT[C/G]ACAATCTCATCATC-3′ for the amplification of genogroup 2 viruses. These primer pairs amplified a fragment of 205 bp corresponding to nucleotides 4674–4878 of Norwalk virus [Jiang et al., 1993]. For genogroup 3 (ClHuCVs), primers 4490 (5′-ACACGTGGTGGTCTACCATCTGG-3′) and 4485 (5′-CACACTGTACATGCA[G/A]TCATCACC-3′) were used; these amplified a fragment of 180 bp corresponding to nucleotides 4345–4524 of Manchester virus [Liu et al., 1995]. For samples containing SRSVs, genogroup 2 primers were used first. If a negative result was obtained, the amplification was repeated with genogroup 1 primers. Genogroup 3 primers were used only with samples identified as containing ClHuCVs by electron microscopy.

DNA Sequencing and Sequence Analysis

Amplified DNA was purified by electrophoresis and sequenced without cloning using the PRISM™ Dye-Deoxy™ Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) as described previously [Cauchi et al., 1996]. All DNA was sequenced on both strands and approximately one-third of isolates were amplified and sequenced more than once. GeneJockeyII software (Biosoft, Cambridge, UK) was used for the initial manipulation of sequences. PHYLIP software version 3.57c was used for phylogeny inference [Felsenstein, 1989]. New sequences of 166, 163, or 133

nt (depending on the primers used) were aligned by CLUSTAL W (1.7) [Higgins et al., 1992] with those of other selected published viruses. Modified data sets (200) were generated by bootstrap resampling and both modified and unmodified data analyzed by DNAPARS or DNADIST and NEIGHBOUR. Consensus phylogenetic trees were derived using CONSENSE and trees drawn using TreeView version 1.4 [Page, 1996]. Although not all minor branch points in trees satisfied the 95% confidence level, the major clusters (genogroups) of isolates were maintained in all trees.

RESULTS

Electron Microscopy

SRSVs were identified as round granular staining particles commonly with a fringe of short spikes [Cauchi et al., 1996]. The number of fecal specimens examined was 6,226. SRSVs were detected in 223 individuals and had a mean diameter of about 35 nm (mean ± S.D. of 34.7 ± 3.2 nm; one particle measured from each of the 223 individuals in which SRSVs were detected). ClHuCVs were identified on the basis of their size and shape, particularly their cup-shaped indentations, as described previously [Madeley and Field, 1988]. The viruses were detected in nine patients and had a mean diameter of about 32 nm (31.6 ± 1.4 nm; one particle measured from each of the nine patients in which calicivirus was detected).

Incidence of SRSVs in Victoria, 1980–1996

One hundred and nineteen incidents of SRSV-associated gastroenteritis were detected in Victoria for the period 1980–1996 (and four in the other two states). For Victoria, the number of incidents varied from year to year, with a small peak in the early 1980s, and increasing virus prevalence during the mid 1990s (Fig. 1B). The number of incidents approximately mirrored the number of fecal samples submitted for testing (Fig. 1A). SRSV-associated gastroenteritis incidents occurred throughout the year, although they tended to occur more frequently in the last two quarters of the year (late winter, spring, early summer) than in the first two (Fig. 1C). This trend was noticeable up to 1994 and was more marked in the large number of incidents in 1995 and 1996.

Classification of Cases and Incidents

Of 154 SRSV cases in people of known age, 16 (10%) were in infants aged 12 months or less; 11 (7%) were in children aged 1 to 15 years; 56 (36%) were in adults aged 16 to 64 years; and 71 (46%) were in elderly persons aged 65 or more. No ages were given in 69 cases. The data indicate that individuals of all ages are susceptible to SRSV-associated gastroenteritis. As sampling tended to be biased toward collecting specimens from adults, the figures do not provide comparative incidence figures for the four age categories. However, for adults, the figures indicate, SRSVs are of particular concern in the elderly.

ClHuCVs were identified in nine individuals; two

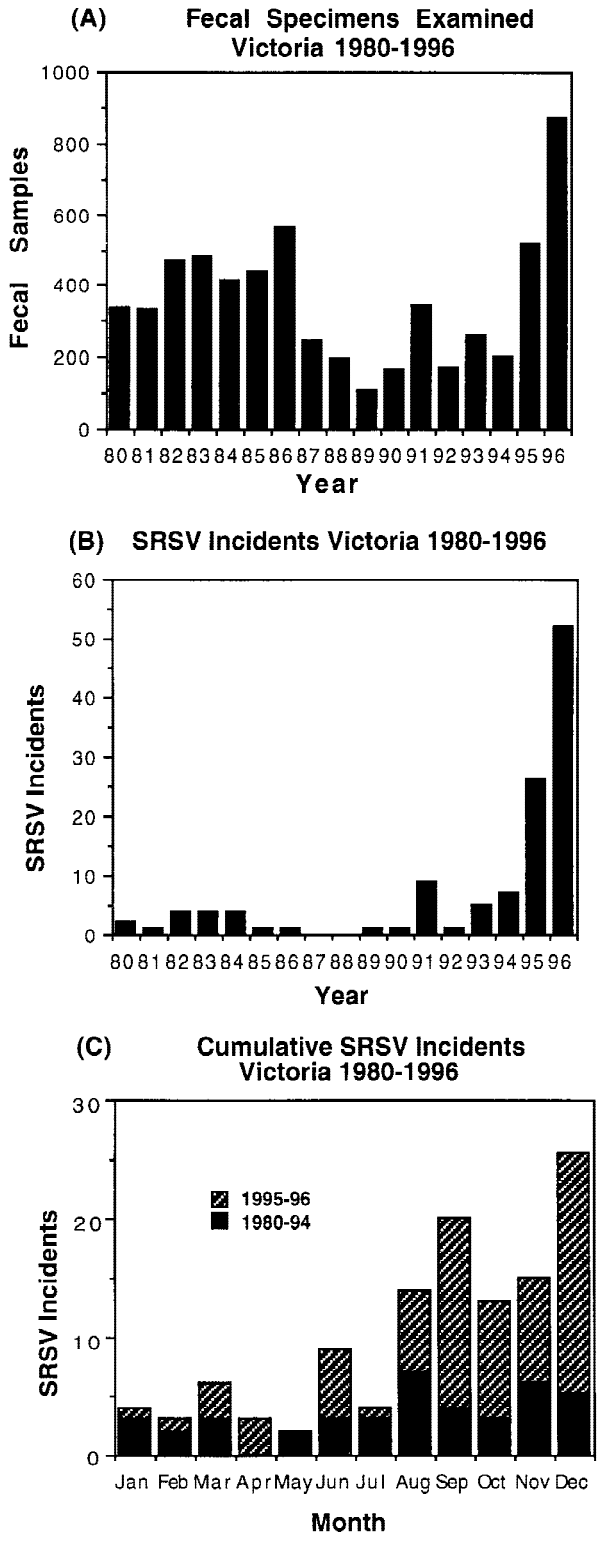

Fig. 1. Annual summaries **(A)** of the fecal specimens examined (total 6,106), and annual **(B)** and monthly **(C)** summaries of SRSV incidents (total 119) in Victoria for the period 1980–1996.

TABLE I. SRSV and ClHuCV Isolates in Southeastern Australia, 1982–1996, Listed by Genogroup With Other Selected Viruses

Tree[a]	Designation	Location[b]	Date, month/year	Patient age and setting (accession number*)		
			Genogroup 1			
1*	Southhampton/91/UK			L07418		
2	035806/96	Unknown	10/96	adult	unknown	sporadic
3*	Desert Shield/90/SAA			U04469		
4*	NV/8FIIa/68/US (Norwalk)			M87661		
5*	KY-89/89/J			L23828		
6*	Sa-1283/84/J			L23832		
7	041884/96	Parkville	12/96	elderly	hospital	outbreak
8	032101/95	Brighton	6/95	adult	reception	outbreak
			Genogroup 2A			
9	G5175/84	Goulburn Valley	84	unknown	reception	outbreak
10	G8357/90	Fairfield	10/90	adult	hospital	sporadic
11	144330/93	Glen Waverley	9/93	unknown	hospital	outbreak
	148852/93[c]	Caulfield	10/93	unknown	restaurant	outbreak
	144306/93	Box Hill	8/93	elderly	unknown	unknown
12*	OTH-25/89/J			L23830		
13*	925/92/UK			L23826		
14*	Mexico 34/89/MEX			U22498		
15*	MV24/91/CAN (Toronto)			U02030		
16	046172/95	Wantirna	8/95	adult	hospital	outbreak
17	042530/95 (Carlton)[d]	Carlton[f]	7/95	infant	hospital	outbreak
	036047/95	Carlton[f]	6/95	infant	hospital	outbreak
	036054/95	Carlton[f]	6/95	infant	hospital	outbreak
	039053/95	Carlton[f]	7/95	infant	hospital	outbreak
	043556/95	Carlton[f]	7/95	adult	hospital	outbreak
	046444/95	Carlton[f]	8/95	infant	hospital	outbreak
	059850/95	Carlton[f]	10/95	infant	hospital	outbreak
18	040935/96	Maryborough[g]	12/96	elderly	restaurant	outbreak
	040937/96	Maryborough[g]	12/96	adult	restaurant	outbreak
	041825/96	Moonambel	12/96	unknown	restaurant	outbreak
19	003714/96	Koo-wee-rup	1/96	unknown	family	outbreak
	033600/96	Benalla	9/96	adult	unknown	sporadic
20	025753/96	Mt Keira NSW	10/96	infant	unknown	sporadic
			Genogroup 2B			
21*	Melksham/89/UK			X81879		
22	041506/96	St Kilda	12/96	elderly	unknown	sporadic
23	041355/96	Caulfield	12/96	elderly	hospital	outbreak
24	038302/96	Boronia	11/96	child	family	outbreak
25	035230/96	Heidelberg	10/96	elderly	hospital	outbreak
26	013179/96	unknown	3/96	infant	unknown	sporadic
27	033961/96	Pakenham	9/96	elderly	nursing home	outbreak
28	014090/96	Macedon Ranges	4/96	infant	unknown	outbreak
	017652/96	Malvern[h]	4/96	elderly	hospital	outbreak
29	033418/96	Geelong	9/96	elderly	nursing home	outbreak
30	041499/96	Ormond	12/96	elderly	nursing home	outbreak
31	041967/96	Rosebud	12/96	adult	hospital	outbreak
32	040465/96	Prahran	12/96	elderly	hospital	outbreak
33	016404/96	Malvern[h]	4/96	elderly	hospital	outbreak
	033124/96	Murrumbeena	9/96	elderly	nursing home	outbreak
	033610/96	Canterbury	9/96	elderly	nursing home	outbreak
	033809/96	Greenvale	9/96	elderly	nursing home	outbreak
	033959/96	Melton	9/96	unknown	reception	outbreak
	036097/96	Maryborough	10/96	elderly	nursing home	outbreak
	037609/96	Ringwood	11/96	infant	hospital	outbreak
	039385/96	Bundoora	11/96	elderly	nursing home	outbreak
34	042204/96	unknown	12/96	adult	unknown	sporadic
35	065716/95	Campbell Town TAS[i]	11/95	elderly	nursing home	outbreak
36	065711/95	Campbell Town TAS[i]	11/95	elderly	nursing home	outbreak
37	138534/94	Frankston	9/94	elderly	nursing home	outbreak
38	G8487/91	Fairfield	6/91	elderly	hospital	sporadic
39	G8520/91	Heidelberg	7/91	adult	hospital	sporadic
40	058430/95	Caulfield	10/95	elderly	hospital	outbreak
41	169506/94	Gisborne	11/94	elderly	nursing home	outbreak
42*	101922/94 (Camberwell)[e]	Camberwell	1/94	elderly	nursing home	outbreak
				U46500		
43*	Lordsdale/93/UK			X86557		
44	G4407/82	Mt Buffalo	82	unknown	chalet	outbreak
45	G4077/82	Geelong	6/82	unknown	hospital	outbreak

(continued)

TABLE I. Continued

Tree[a]	Designation	Location[b]	Date, month/year Genogroup 1	Patient age and setting (accession number*)		
46	024477/96	Box Hill	6/96	elderly	hospital	outbreak
47	172631/94	Parkville[j]	12/94	elderly	hospital	outbreak
	172632/94	Parkville[j]	12/94	elderly	hospital	outbreak
48*	Hawaii/71/US			U07611		
49	G8512/91	Malmsbury	7/91	unknown	camp	outbreak
50	G8673/91	Beechworth	11/91	elderly	nursing home	outbreak
51*	Snow Mt Agent/76/US			L23831		
52	017680/96	Box Hill[k]	4/96	adult	reception	outbreak
53	0177648/96	Box Hill[k]	4/96	adult	reception	outbreak
	017649/96	Box Hill[k]	4/96	adult	reception	outbreak
			Genogroup 3			
54	G8287/90	Fairfield	5/90	adult	hospital	sporadic
55*	Plymouth/92/UK			X86559		
56	G3965/82	Prahran	4/82	adult[l]	hospital	outbreak
57*	Sapporo/DCC/86/US (Houston)					
58*	Sapporo/82/J			S77903		
59	G5488/84	Fairfield	6/84	adult	hospital	sporadic
60	015894/96	unknown	4/96	infant	unknown	sporadic
	027894/96	unknown	6/96	child	unknown	sporadic
61*	Manchester/93/UK			X86560		
62	013959/96	unknown	3/96	child	unknown	sporadic
	014229/96	unknown	3/96	infant	unknown	sporadic
63	007778/96	unknown	2/96	infant	unknown	sporadic
64*	Parkville			U73124		
65*	Vanderbijlpark/313616/93/SA			U43287		
66*	Pretoria/MK17/94/SA			U50825		
67*	Pretoria/205430/92/SA			U50824		

[a]Reference number in phylogenetic tree (Fig. 2). Asterisk denotes published sequence. Genogroup 1: Norwalk/Southhampton-like; minimum pairwise identity: nucleotide 69.9%, amino acid 86.5%. Genogroup 2A: Mexico/Toronto-like; minimum pairwise identity: nucleotide 77.3%, amino acid 92.6%. Genogroup 2B: Lordsdale/Camberwell-like; minimum pairwise identity: nucleotide 77.1%, amino acid 92.7%. Genogroup 3: Sapporo/Manchester-like (classic human calicivirus); minimum pairwise identity: nucleotide 63.2%, amino acid 77.3%.
[b]Isolates were obtained within Victoria unless indicated.
[c]Isolates of identical sequence are indented under the isolate represented in Figure 2.
[d]Carlton outbreak described in Marshall et al. [1997].
[e]Camberwell outbreak described in Cauchi et al. [1996].
[f–k]Isolates from the same outbreak.
[l]Matson et al. [1995].

were infants, three were children, three were adults, and one was of unknown age. ClHuCVs were not detected in any elderly person. Taken together with the SRSV data, the results suggest that ClHuCVs are not a major concern for the elderly.

Incidents involving viruses of either morphological type were classified as either an outbreak or as a sporadic incident (see Materials and Methods). In a total of 123 SRSV-associated incidents, 70 were classified as an outbreak and 53 as sporadic. In the nine incidents where ClHuCVs were detected, one was classified as an outbreak and eight as sporadic. The data indicate that incidents of SRSV-associated gastroenteritis quite commonly occur as an outbreak; the data on ClHuCVs are limited, but suggest that sporadic cases are common forms of manifestations of this virus.

SRSV incidents were classified as occurring in one of five settings: (i) in a hospital; (ii) in a nursing home; (iii) associated with a restaurant or reception; (iv) in a camp, chalet, or child care center; or (v) within a family group or of uncertain setting. The relative frequencies of these settings for the 119 Victorian SRSV incidents were: (i) 36%; (ii) 19%; (iii) 13%; (iv) 5%; and (v) 26%. The number of outbreak and sporadic incidents in each setting were: (i) 23, 20; (ii) 23, 0; (iii) 16, 0; (iv) 6, 0; and (v) 1, 30, respectively. Thus, SRSV incidents are of major concern in hospitals and nursing homes (together accounting for 55%). For the nine ClHuCV incidents, three were referred by hospitals and six were of uncertain origin.

Sequencing and Genogroups

Table I lists the 69 isolates that were sequenced over a segment of ORF1 (putative RNA polymerase region). These isolates represent 61/223 and 8/9 of the fecal specimens positive by electron microscopy for SRSVs or ClHCuVs respectively, and 57/132 of all incidents. The sequenced isolates were selected from 126 specimens positive by RT-PCR, and their distribution across patient age groups and settings reflected the distribution in the total of 232 isolates and 132 incidents. For this study, it was not considered necessary to pursue the reasons for lack of amplification in the RT-PCR-negative specimens. Many had been stored for long periods (the earliest sample amplified was from 1982) and sufficient positives were available for sequencing. Forty-six sequences were unique; this total included Camberwell virus (#42). The isolates have been numbered for cross-referencing to Figure 2. Selected pub-

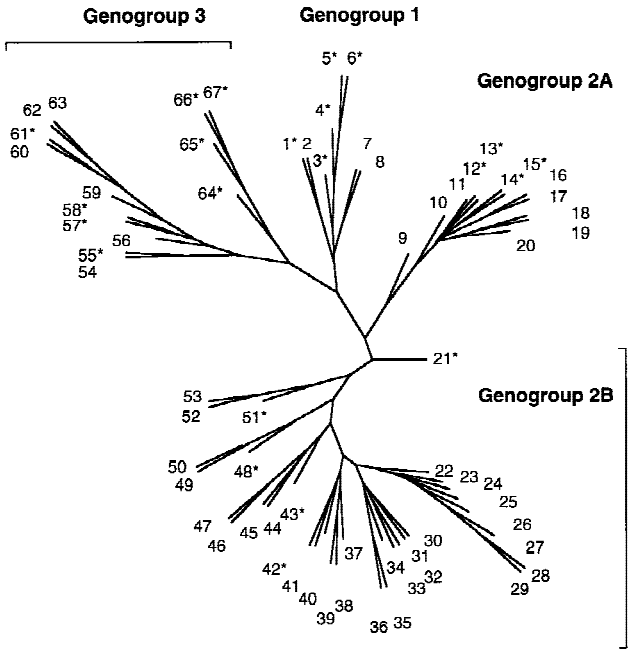

Fig. 2. An unrooted phylogenetic tree generated by the parsimony method using nucleotide sequences. The radial tree was drawn with TreeView. The isolate numbers correspond to those listed in Table I. Published sequences are indicated with an asterisk.

lished sequences were added to the list to provide comparisons with the Australian isolates.

The list in Table I contains six examples of outbreaks, where more than one fecal sample was examined. The most interesting of these (Carlton, #17) occurred over several months, predominantly in babies less than eight months old, and has been reported previously [Marshall et al., 1997]. No nucleotide sequence variation was detected in the ORF1 segment. Likewise, no sequence variation was detected in the Maryborough (#18) or Parkville (#47) outbreaks. However, there were differences in sequence in the Malvern (#28, #33), Campbell Town (#35, #36), and one of the Box Hill (#52, #53) outbreaks. These differences were limited to two positions in a segment of 163 nt. This suggested that the variation was introduced by viral RNA polymerase during viral replication, or by errors during RT-PCR, rather than by viruses from more than one source.

The viruses described in Table I clustered into several genogroups when the nucleotide sequences were used to generate phylogenetic trees by parsimony or distance matrix methods. Identical groupings were obtained by the two methods. One such unrooted tree is shown in Figure 2, where the radial presentation facilitates the identification of clusters. All viruses identified by electron microscopy as SRSVs belonged to genogroup 1 or 2 , and all ClHuCVs belonged to genogroup 3. No examples of ClHuCVs falling into genogroup 1 or 2 were noted [Cubitt et al., 1994; Stene-Johansen and Grinde, 1996]. Pairwise comparisons of nucleotide identity between all isolates are not listed because of the large number; however, some examples using viruses at the extremities of the tree are given (Table II). In addition, minimum identities within a genogroup are shown in Table I.

A consensus for the deduced amino acid sequence of each genogroup was determined (Fig. 3). The motif GLPSG was present in all SRSVs. The common YGDD polymerase motif was coded in part by the downstream primer and is partially represented (YG) at the carboxy termini of the consensus sequences in Figure 3. For genogroups 1, 2A, and 2B, the consensus amino acids were strongly supported in the majority of positions where variation was noted, i.e., the alternative amino acid shown was infrequent. Thus, once the deduced amino acid sequence for a new virus was known, an immediate provisional assignment of the virus to a genogroup was possible without further phylogenetic analysis. For example, the viruses belonging to the genogroup 2 subgroups could be distinguished from each other by the amino acids underlined in Figure 3. As previously reported, the classical human caliciviruses (genogroup 3) have a unique sequence with an insert of five amino acids that is easily recognized [Lambden et al., 1994]. The alternative amino acids shown for genogroup 3 in Figure 3 were due almost entirely to variation in published sequences; the new isolates described here differed at only one position (the last isoleucine residue in Fig. 3).

DISCUSSION

Clusters or genogroups similar to those reported above and based on ORF1 sequences have been described by others, although there is no universal agreement on the nomenclature [Jiang et al., 1996; Berke et al., 1997; Hardy et al., 1997]. Genogroup 1 contained the fully sequenced Norwalk and Southampton viruses [Jiang et al., 1993; Lambden et al., 1993]. To avoid confusion with the more general and historical term "Norwalk-like" particles or viruses, we refer to this group as Norwalk/Southampton-like. Genogroup 2, also known as the Snow Mountain-like group, clearly contained two subgroups [Jiang et al., 1996]. Mexico and Toronto viruses are typical of the first subgroup, while Lordsdale and Camberwell viruses are typical of the second subgroup. Considerable sequence information is available for these four viruses and they have been frequently used in published comparisons between viruses [Lew et al., 1994; Cauchi et al., 1996; Jiang et al., 1996; Green et al., 1997]. For convenience, the subgroups are referred to here as 2A and 2B, terms that are consistent with the P2-A and P2-B designations used by Ando et al. [1995].

Genogroup 3 contained caliciviruses with typical or classical calicivirus morphology, whereas the other genogroups contained only SRSVs. Sapporo calicivirus has historically been regarded as typical of this group, and the full genome sequence of Manchester virus is known [Liu et al., 1995]. Therefore, we use the name Sapporo/Manchester-like for this genogroup. The variation within this genogroup (minimum 69.9% nucleotide identity, Table I) over the ORF1 fragment

TABLE II. Percentage Identities in Pairwise Nucleotide Comparisons Between Selected Sequences

		Genogroup											
		1		2A		2B					3		
Tree[a]	Virus	4*	6*	15*	16	21*	28	42*	51*	53	61*	63	67*
4*	Norwalk	+	86[b]	56	57	60	59	59	57	55	31	31	29
6*	Sa-1283/84/J		+	54	53	63	59	58	54	57	31	31	26
15*	Toronto			+	98	72	76	76	78	78	25	23	22
16*	046172/95				+	72	76	76	79	77	23	23	23
21*	Melksham					+	79	77	77	79	25	26	21
28	014090/96						+	95	83	84	25	26	22
42*	Camberwell							+	83	83	23	26	21
51*	Snow Mt Agent								+	88	23	23	22
53	0177648/96									+	25	27	24
61*	Manchester										+	98	69
63	007778/96											+	68
67*	Pretoria/205430/92/SA												+

[a]Reference number in the phylogenetic tree (Fig. 2 and Table 1).
[b]Underlined percentages indicated comparisons within a genogroup.

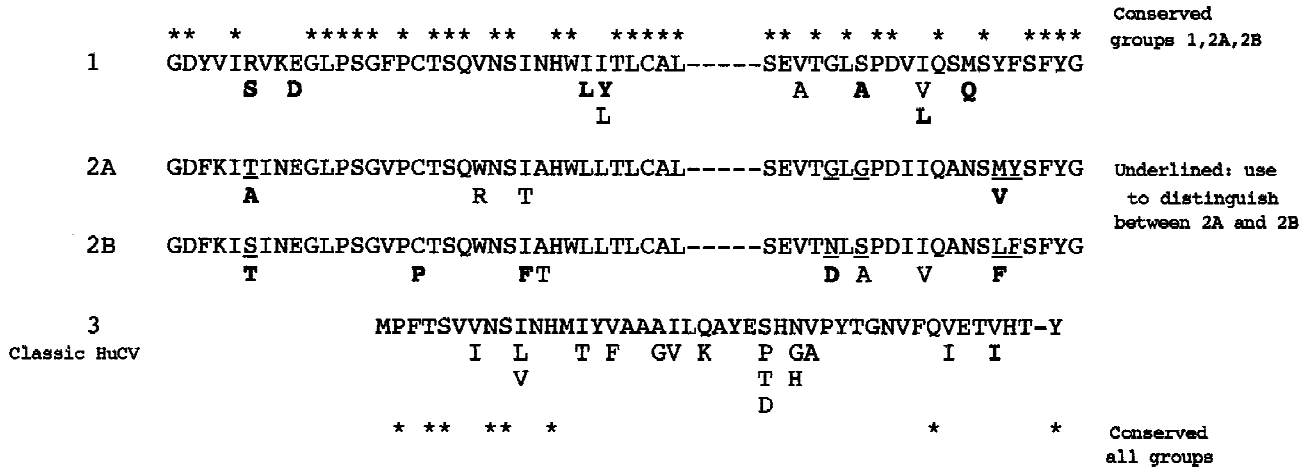

Fig. 3. The consensus-deduced amino acid sequences for genogroups. Amino acids not fitting the consensus are shown underneath the relevant sequence; those in bold correspond to alternatives observed in this study; those in plain text are variations in published sequences (viruses with asterisks are in Table I and Fig. 2).

analyzed was larger than that for any other group, and the tree branching (Fig. 2) indicated more than one subgroup, as suggested by Noel et al. [1997]. Additional sequences obtained for strains Houston/86, Houston 90, and London/92 have also demonstrated considerable diversity within this genogroup [Jiang et al., 1997]. However, the six unique sequences determined in this study (#s 54, 56, 59, 60, 62, 63) were not highly diverse; they showed minimum pairwise identity of 85% (nucleotides) between themselves, had high conservation of deduced amino acid sequence, and were all located in the same region of the tree (Fig. 2) as Sapporo and Manchester viruses.

Melksham virus (#21) has been described as a novel antigenic variant [Green et al., 1995] and occupied a unique position in all ORF1-based phylogenetic trees produced in our analyses. It was clearly in genogroup 2, although it was assigned to the Lordsdale/Camberwell genogroup based on the ORF1 partial sequence. Comparisons of the combined ORF3 and the 3′ untranslated region demonstrated that these parts of the genome were marginally more closely related to Mexico virus (53% nucleotide identity) than to Lordsdale virus (50% nucleotide identity). For ORF2, Melksham virus is more closely related to Snow Mountain virus (94%) and Hawaii virus (65%) than to Mexico (56%) or Lordsdale (55%) viruses (analyses not shown). Thus, Melksham virus illustrates the limitations of subdividing genogroup 2 into two groups based on a short segment of ORF1. The division is a useful one at this stage; although it needs support from further sequencing of ORF1 and the other ORFs, we believe it is justified because the major SRSV genogroups 1 and 2 are maintained across ORF1, ORF2, and ORF3, where the additional sequence information is available [Lew et al., 1994; Cauchi et al., 1996; Green et al., 1997]. Further work is also needed to reconcile the several SRSV antigenic typing schemes with genogroups [Okada et al., 1990; Lambden et al., 1993; Lewis et al., 1995; Hardy et al., 1997]. Antigenic types depend on the capsid protein and therefore the ORF2 sequence. It will be possible to relate antigenic types to ORF1 genogroups only if there

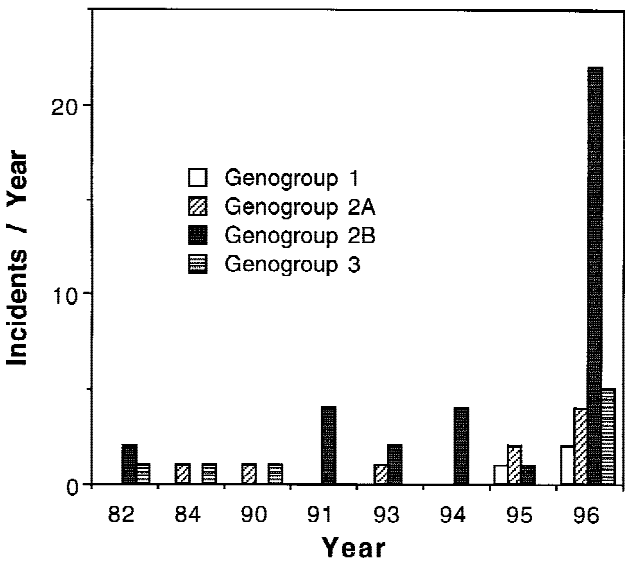

Fig. 4. Analysis by year of genogroups detected in Victoria, 1982–1996.

is a nexus between ORF1 and ORF2 sequences [Berke et al., 1997].

Since 1982, the majority of SRSVs detected in southeastern Australia belonged to genogroup 2B, particularly in the 1990s (Table I and Fig. 4). From Table I, it can be seen that the majority of group 2B incidents were in elderly people in the setting of a nursing home or a hospital. Viruses of this subgroup were the main contributors to the large number of incidents in 1996. Group 2A viruses were also detected throughout the years, whereas genogroup 1 viruses were rare and only detected in 1995/1996. The dominance of group 2 viruses (subgroup unknown) in the 1980s and 1990s has also been noted in South Africa and England [Caul, 1996a; Cubitt and Jiang, 1996; Wolfaardt et al., 1997], as well as in the Netherlands in 1994/1995 [Vinje and Koopmans, 1996]. However, genogroup 1 viruses continue to circulate and remain a cause of SRSV-associated disease [Stene-Johansen and Grinde, 1996; Vinje and Koopmans, 1996; Gray et al., 1997].

The year-by-year increase in all SRSV incidents since 1992 in Victoria (Fig. 1B) parallels the increase in reports of SRSVs in England and Wales during 1990–1995 [Caul, 1996b: Hale, 1997]. In Victoria, incidents were most frequent around spring time in the third and fourth quarters of the year, particularly in 1995 and 1996 (Fig. 1C), whereas in England and Wales the first and fourth quarters corresponding to winter showed peak SRSV activity [Caul, 1996b]. The reasons for the seasonal differences between countries and hemispheres are unknown.

This study, one of the most detailed long-term overviews of SRSVs in a given locale, emphasizes the public health significance of the viruses in the institutionalized and the elderly in particular. The continuing molecular characterization should point the way to developing vaccine strategies in high-risk groups.

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